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Diversity of indoor fungi as revealed by DNA metabarcoding

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Abstract: In the present study, we conducted DNA metabarcoding (the nuclear ITS2 region) for indoor fungal samples originating from two nursery schools with a suspected mould problem (sampling before and after renovation), from two university buildings and from an old farmhouse. Good-quality sequences were obtained, and the results showed that DNA metabarcoding provides high resolution in fungal identification. The numbers of fungal classes, orders, families and genera per sample varied greatly among sampling sites (pooled results per building) and times, between 12-21, 15-58, 20-118 and 29-248, respectively. Comparable ranges of Shannon's diversity indices were 0.47-2.12, 0.65-2.91, 0.82-3.30 and 0.87-3.59, respectively. The pooled proportions of filamentous ascomycetes, filamentous basidiomycetes, yeasts and other fungi equalled 62.3%, 8.0%, 28.3% and 1.4%, respectively, and the total number of fungal genera found during the study was 585. When comparing fungal diversities and taxonomic composition between different types of buildings, no obvious pattern was detected. The average pairwise values of $Sørensen_{Chao}$ indices that were used to compare similarities for taxon composition between samples among the samples from the two university buildings, two nurseries and farmhouse equaled 0.693, 0.736, 0.852, 0.928 and 0.981, respectively, while the mean similarity index for all samples was 0.864. We discovered that making explicit conclusions on the relationship between the indoor air quality and mycoflora is complicated by the lack of appropriate indicators for air quality and by the occurrence of wide spatial and temporal changes in diversity and compositions among samples.

Key words: indoor air quality, fungi, metabarcoding, next generation sequencing

Introductiom

Environmental microbes can have both beneficial and harmful effects on health, and the interactions between environment, microbiota and health may be complicated. For instance, biodiversity of bacteria is believed to be an important factor explaining the lower incidence of allergic diseases in children living in high-biodiversity conditions (rural environments) when compared to children living in urban environments with lower biodiversity (Hanski et al. 2012; Ruokolainen et al. 2015). Such an environmental effect may be mediated via the effect of environmental microbiota on the commensal microbiota influencing immunotolerance. Based on the proposed biodiversity hypothesis, early exposure to an environment with high biodiversity may prevent the development of allergic diseases (Hanski et al. 2012; Ruokolainen et al. 2015).

People spend most of their time in indoor environments, which contain a variety of microbes. Serious problems may develop in buildings with long-lasting dampness, where the moisture supports the growth of bacteria and fungi (i.e., mould). Based on epidemiological studies, mould in buildings is positively associated with several allergic and respiratory effects, and certain moulds are toxigenic, meaning that they can produce mycotoxins (Fisk et al. 2007; Mendell et al. 2011; Jacobs et al. 2014). There are estimates that allergic diseases caused by plant, animal and fungal allergens affect more than 30% of the population in industrialized countries (Crameri et al. 2013), and there is increasing awareness and concern over exposure to moulds in indoor environments. The phenomenon has become known as Sick Building Syndrome (SBS), where the occupants describe a complex range of vague and often subjective health complaints (Jones 1999). Since a presumed mould problem may lead to expensive renovations or even to the abandonment of buildings, it is important to be able to evaluate the mould situation, as well as the potential presence of other indoor air pollutants, correctly and precisely.

Indoor fungi are traditionally determined by culture-dependent methods (e.g. Ebbehøj et al. 2002), which have a low taxonomic resolution, underestimate diversity, and bias results towards fungi that grow well on generic growth media and produce characteristic morphological structures allowing identification. In fact, there are many cryptic fungal species that cannot be distinguished morphologically or based on reproductive characteristics (Sato and Murakami 2008; Brown et al. 2013). Presently, taxon-specific microbial markers combined with quantitative PCR methods are also used for identifying fungal specimens (e.g. Simoni et al. 2011; Jacobs et al. 2014).

Recent advances in DNA sequencing provide an effective tool for species detection and biomonitoring using DNA present in the environment. Specifically, DNA metabarcoding through high-throughput sequencing (next generation sequencing) allows the characterization of the species composition of bulk samples, including both intact and degraded DNA extracted from environmental samples (eDNA, i.e., cellular DNA from living cells or organisms and extracellular DNA resulting from cell death and subsequent destruction of cell structure) (Taberlet et al. 2012; Bohmann et al. 2014; Yang et al. 2014; Valentini et al. 2015). Metabarcoding uses universal PCR primers to mass-amplify a taxonomically informative gene from mass collections of organisms or from environmental DNA.

In the present study, to increase precision in analyses and to provide useful data and tools for end-users on the environmental quality of indoor spaces, and to discover existing biodiversity in indoor fungal communities, we conducted DNA barcoding (the nuclear ITS2 region) for indoor fungal samples. The internal transcribed spacer region (ITS, comprising spacers ITS1 and ITS) of the nuclear ribosomal DNA is the formal DNA barcoding region for molecular identification of fungi (Schoch et al. 2012). It has been shown that ITS1 and ITS2 yield closely similar results when used as DNA barcodes for fungi (Blaalid et al. 2013). Thus, the use of ITS2 in fungal metabarcoding is justified. In this study, we wanted to test, how effective DNA

barcoding is when analysing the taxonomic diversity of fungal communities in indoor spaces. The additional novelty was that we collected samples from different types of buildings and included multiple samples from each building at different time points (i.e., a longitudinal study approach). Two buildings were sampled both before and after renovation in order to discover, whether the renovation affected the fungal composition.

Materials and methods

Samples were collected from five buildings, including two university buildings, two nursery schools and an old inhabited farmhouse (field crop production nearby). The farmhouse is located in the county of Porvoo, about 40 km to the east from Helsinki, while other buildings are located in Helsinki (about 60°14' N, 25°01' E). Sampling was conducted four times: January 2013, July 2013, January 2014, and July 2014. All buildings were not sampled on every occasion (Table 1). Both nursery schools were renovated during the study due to respiratory symptoms reported by some employees and minor visible water damage and mould growth, and we sampled them both before and after renovation, which primarily included changes in surface materials and improved ventilation. Sampling was performed using a collector with a disposable filter (DUSTREAM Collector, Indoor Biotechnologies Inc., Charlottesville, VA, USA; mesh size 40 µm) attached to the tube of a vacuum cleaner with the suction power of 32 L/s. Both a horizontal (tables or shelves) and vertical (walls) sample were collected by vacuuming an area of about 2 m^2/sample (i.e., two 2 m^2 samples per room) from two rooms in each of five buildings (two office rooms in each university building, two playrooms in each nursery school, and two bedrooms in the old farmhouse; nursery schools and the farmhouse had suspected mould problems).

After vacuuming, the filter containing the dust was removed from the collector and placed in a plastic bag until processing. In a fume hood in the lab, filters were cut, rinsed with water, and the dust and water were poured into a petri dish, where large non-biological particles were removed. Thereafter, the samples were dipped in liquid nitrogen and ground in a ball mill, and DNA was extracted using the CTAB (cetyl trimethylammonium bromide) method (Doyle and Doyle 1987). The final volume was 100 µl.

For the metabarcoding of the fungal samples, genomic ITS2 sequences were amplified and sequenced using two approaches. All sequencing was conducted at the DNA Sequencing and Genomics Laboratory, Institute of Biotechnology, University of Helsinki. The sequencing for the samples from January and July 2013 were conducted using 454 FLX pyrosequencing (Roche Applied Science, Penzberg, Germany), as described in Korpelainen et al. (2015). The following primer systems were used:

1) Reverse *ITS3_Ampl_B* (adapter + ITS3 primer) [the same one in all reactions]

5'-CTATGCGCCTTGCCAGCCCGCTCAG + GCATCGATGAAGAACGCAGC-3'

2) Forward *ITS4_Ampl_A+Tg* (adapter + tag marker (6 bp) + ITS4 primer) [different tag marker alternatives], for instance

5'-CGTATCGCCTCCCTCGCGCCATCAG + TCTGTA + TCCTCCGCTTATTGATATGC-3'

The used tag marker sequences were as follows: TCTGTA, CTACTG, CAGCTC, ATCATG, AGATAT, CGACGC, CATGCA and TCTATG.

However, sequencing for the samples from January and July 2014 were performed using Illumina MiSeq sequencing (San Diego, CA, USA), for which ITS2 sequences were first amplified using the following primer system (forward ITS4 mix + reverse ITS3 mix):

Forward ITS4 mix including three primers:

ITS4_F1 5'-ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCTCCGCTTATTGATATGC-3’

ITS4_F2 5'-ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT(c/g)TCCTCCGCTTATTGATATGC-3

ITS4_F3 5'-ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTagt(a/g)(a/g)TCCTCCGCTTATTGATATGC-3’

Reverse ITS3 mix including three primers:

ITS3_R1 5’-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATCGATGAAGAACGCAGC-3’

ITS3_R2 5’-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT(c/t)GCATCGATGAAGAACGCAGC-3’

ITS3_R3 5’-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTa(a/t)GCATCGATGAAGAACGCAGC-3’

All 20-µl PCR reactions contained 2 µl of template DNA, and the concentration of each primer was 0.25 µM. All PCR products were gel-purified (Omega Bio-Tek Gel Extraction Kit, Norcross, Georgia, USA). After Illumina sequencing, primer sequences were removed from the raw reads, and quality control, as described by Brown et al. (2013), followed. During this process, low-quality reads (below average PHRED score of 25) and short sequences (< 100 bp) were removed. Then, all other sequence data were subjected to similarity search against GenBank (www.ncbi.nlm.nih.gov/genbank), and assignment of taxonomic identities using TAXAassign (https://github.com/umerijaz/ taxaassign) was conducted with 60, 70, 80, 95, 95, and 97% thresholds for different taxonomic ranks, which may correspond to phylum, class, order, family, genus and species levels, respectively. However, these thresholds are tentative and should be treated with special caution, except for the 97% threshold, which is, by convention, used as a divergence threshold for operational taxonomic units (OTUs) that serve as a proxy for species (Brown et al. 2015), The sequence data were submitted to the EMBL (European Molecular Biology Laboratory) database under accession number PRJEB8345. Based on the numbers of sequences representing each taxon (i.e., taxon distribution), Shannon’s diversity

indices (Shannon 1948) were calculated at class, order, family, and genus level for each sample. To compare the taxonomic composition of pooled samples (each including all four samples from a building at the same time point), a principal component analysis (PCA) was conducted for the ITS2 sequence-based generic data (fungal genera and their frequencies) using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). In addition, EstimateS 9.1.0. (http://purl.oclc.org/estimates) was used to calculate similarities for taxon composition between pooled longitudinal samples from the same site and between all pooled samples. The used estimator was the $Sørensen_{Chao}$ abundance-based similarity index (corrected for unseen shared species), which can also handle different sample sizes (Chao et al. 2005).

Results

Relatively small-scale pyrosequencing was conducted for the first two sets of samples (winter 2013 and summer 2013), and the number of good sequences averaged 6569 and 4967 sequences/sample (original 2 m^2 sample), respectively. For the last two sets of Illumina-sequenced samples (winter 2014 and summer 2014), the number of good sequences averaged 213 894 and 558 756 sequences/sample (original 2 m^2 sample), respectively. Of all samples, 85.8% were successfully assigned to the genus level and 49.0% to the species level. We present diversity and taxonomic information based on genus level data unless otherwise specified.

Fungal taxa per sample varied greatly among sampling sites (pooled results per building) and times. Based on 454 FLX pyrosequencing (January and July 2013 samples), numbers of taxa were as follows; 12-21 classes, 15-58 orders, 20-114 families, and 29-176 genera, while based on Illumina MiSeq sequencing (January and July 2014 samples), the numbers were as follows: 15-19 classes, 46-58 orders, 82-118 families, and 144-248 genera (tentative classification; Table

1). Comparable ranges of Shannon's diversity indices for 454 FLX pyrosequenced data were 0.88-2.12, 1.09-2.91, 1.18-3.30 and 1.18-3.59, respectively, and for Illumina MiSeq data 0.47-1.87, 0.65-2.57, 0.82-3.00, and 0.87-3.52. The total number of fungal genera found during the study was 585. The change of the sequencing method did not result in increased diversity indices (genus level, determined for individual 2 m^2 samples, mean±standard error; 2.79±1.00 in 2013 and 2.56±0.94. However, there was an increase in the numbers of detected taxa, with a mean of 89.1±57.7 genera in 2013 and 122.4±57.7 genera in 2014 ($t=2.627$, $df=54$, $P=0.012$). Thus, apparently a more comprehensive coverage of low-frequency taxa was obtained using Illumina sequencing. The numbers of taxa and diversity indices were similar among individual horizontal (100.2±41.2 genera; 2.83±0.65) and vertical (105.7±56.7 genera; 2.55±1.16)and, and among winter (105.2±51.1 genera; 2.58±1.11) and summer samples (102.5±44.6 genera; 2.77±0.88). In Nursery 1, which underwent a small-scale renovation, the diversity index did not change, while in Nursery 2, which was renovated extensively, the diversity index increased from 1.18 to 3.07.

When the fungal taxa detected in each sample were divided into four groups, filamentous ascomycetes, filamentous basidiomycetes, yeasts, and other fungi, the results showed great variation in proportions among sampling sites and times (Fig. 1), and no detectable patterns among samples within and between buildings were found. The proportions of sequences corresponding to filamentous ascomycetes, filamentous basidiomycetes, yeasts, and other fungi were 62.3%, 8.0%, 28.3% and 1.4%, respectively.

Table 2 lists the five most frequent fungal taxa detected in each building at each sampling time. Besides genus, the species name is given in the case of a species-level identification. The results show that there was a seasonal turnover in the proportions of dominant taxa, except for the farmhouse, in which *Cyberlindnera jadinii* and *Candida* sp. were the two most frequent taxa at both sampling times (summer 2013 and winter 2014). *Aureobasidium pullulans* was in the top five taxa in 9 out of 16 building samples, *Cladosporium* sp. in 7

samples, and *Cryptococcus* sp. and *Saccharomyces cerevisiae* in 6 samples each (Table 2). In several samples, one specific taxon highly dominated, such as *S. cerevisiae* in University 1 and Nursery 2 in winter 2013 (47.5% and 45.4%, respectively), *C. jadinii* in the farmhouse in summer 2013 (38.7%), and *Preussia* sp. in University 1 in summer 2014 (85.7%). Overall, the most frequent taxa were *A. pullulans* (10.5%) and *S. cerevisiae* (7.8%).

The taxonomic content of the 14 pooled fungal samples, based on the ITS2 sequence data, was analyzed using principal component analysis (PCA) (Fig. 2). Two components explained 57.7% of the variability. This projection of taxonomic data also confirmed the presence of a great temporal turnover in the composition of samples, except for the two pooled farmhouse samples (F-S13, summer 2013; F-W14, winter 2014). Otherwise, Fig. 2 does not show any seasonal pattern or any definite pattern in the fungal composition of different types of buildings. Correspondingly, $Sørensen_{Chao}$ indices that were calculated to compare similarities for taxon composition between samples did not show any clear pattern. The average pairwise values for temporal pooled samples among University 1, University 2, Nursery 1, Nursery 2 and farmhouse samples equaled 0.693, 0.736, 0.852, 0.928 and 0.981, respectively, while the mean similarity index for all samples was 0.864. The similarity indices of Nursery 1 and Nursery 2 samples for before and after renovation samples equaled 0.739 and 0.928, respectively. For comparison, Shannon's genus-level diversity indices of Nursery 1 equaled 3.42 and 3.41 before and after renovation, and those of Nursery 2 equaled 1.18 and 3.07, respectively.

Discussion

The present study shows that DNA metabarcoding gives a good resolution in fungal identification. The used method is highly effective until the genus level identification of fungi

(85.8%) and reasonably effective for species identification (49.0%). The change of sequencing method from 454 FLX pyrosequencing to Illumina MiSeq sequencing and resulting 70-fold increase in sequence numbers are suggested to increase detection of infrequent taxa, with the mean number of genera per building increasing from 89 to 122. However, year-to-year variation may also contribute to changes in taxon numbers. Considering sequencing platform qualities, Kozich et al. (2013) have demonstrated that Illumina MiSeq platform can provide data that are at least as good as that generated by the 454 platform while providing considerably higher sequencing coverage at a lower cost. Previously, Pitkäranta et al. (2011) have shown that molecular profiling may reveal a five to ten times higher diversity at the genus level than culture-based methods. However, we do not know what fungal diversity culture-dependent methods might reveal in the buildings studied here.

Fungal diversities in samples collected from different buildings (university offices, nursery schools, farmhouse, supposed with or without a mould problem), during different seasons (summer vs. winter) or using different sampling methods (horizontal vs. vertical surface) showed considerable variation and turnover but no definite pattern. Previously, Adams et al. (2013) surveyed temporal variation in airborne fungal assemblages, both indoors and outdoors, using ITS1 pyrosequencing. They discovered that indoors fungal assemblages were diverse and strongly determined by dispersal from outdoors, and no fungal taxa were found as indicators of indoor air quality. Also, human occupancy has been shown to result in significantly elevated airborne bacterial and fungal concentrations as compared to vacant conditions (Hospodsky et al. 2015).

The considerable variation in fungal composition found to occur even within the same building emphasizes the importance of multiple sampling. Also, a diverse array of fungi occurred even in a normal indoor environment (recently renovated University 1 and relatively new University 2) considered to have a good indoor air quality. Nursery 1 and Nursery 2, with some

moisture damage and employees suffering from possibly mould-related symptoms, possessed highly divergent diversity indices before renovation, 3.41 and 1.18, respectively, but similar values after renovation, 3.41 and 3.07, respectively. The $Sørensen_{Chao}$ similarity index for before and after comparisons of taxon composition was lower for Nursery 1 (0.739) than for Nursery 2 (0.928). Cleaning frequency and coverage in different buildings may also contribute to the observed fungal diversities, although all studied buildings are cleaned regularly. When examining fungal diversity by metabarcoding using ITS1 in different apartments in South Korea, An and Yamamoto (2016) observed that Shannon diversity indices were variable but quite low, ranging from 0.14 to 2.29 (mean = 1.11) in indoor spaces considered alike. In addition, renovation may not instantly affect the fungal and bacterial composition, as shown by Emerson et al. (2015), who compared flood-damaged and non-flooded homes. The flooded homes had higher fungal abundances, and the bacterial and fungal communities continued to be affected by flooding, even after relative humidity had returned to baseline levels and remediation had removed any visible evidence of flood damage.

The presence or dominance of fungal taxa known to cause allergic and respiratory effects and/or being indicators of moisture damage could show something of the air quality. Of the 78 fungal genera listed by Simon-Nobbe et al. (2007) to have been shown to induce allergies in atopic (hypersensitive to allergens) individuals, 51 (65%) were found in this study, although most at very low frequencies. Among them, 11 genera were found at the frequency of more than 1% in the whole data set, namely the filamentous ascomycetes *Aspergillus, Aureobasidium, Chaetomium, Cladosporium, Epicoccum, Leptosphaeria* and *Penicillium*, and the yeasts *Candida, Malassezia, Rhodotorula* and *Saccharomyces*. Previously, An and Yamamoto (2016) found several allergy-related genera in apartments in South Korea, where the most abundant genera were *Cladosporium, Crivellia, Rhodotorula* and *Alternaria*.Among university samples, the most common fungal taxa were the filamentous ascomycetes *Aureobasidium pullulans* and

Preussia sp., and the yeasts *Saccharomyces cerevisiae*, *Cyberlindnera jadinii*, *Rhodotorula* sp. and *Cryptococcus* sp., of which *A. pullulans*, S. *cerevisiae* and *Rhodotorula* are listed among the allergy-inducing fungi (Simon-Nobbe et al. 2007). Among nursery school samples, the most common taxa were filamentous ascomycetes *Cadophora*, *A. pullulans* and *Pestalotiopsis*, and the yeast *S. cerevisiae* and *Cryptococcus* sp., of which *A. pullulans* and *S. cerevisiae* are presumed to induce allergies (Simon-Nobbe et al. 2007). In the farm samples, the yeasts *C. jadinii* and *Candida* sp. were the dominant taxa. Among these fungi, *Candida* may cause clinically significant infections (Simon-Nobbe et al. 2007). We also determined the percentages of sequences representing presumably allergy-inducing fungi in each of the 14 pooled samples based on the classification of Simon-Nobbe et al. (2007). The percentages ranged among university samples between 8-81%, among nursery schools before and after renovation between 32-70% and 54-56%, respectively, and in the two farmhouse samples the proportions equalled 54 and 56%. Thus, no obvious pattern was detected. Yet, the view of the nursery school having a mould problem may be subjective, as there has not been a proper medical examination for its users. Also in previous studies, which have been generally based on a narrower range of information, there have been difficulties in the interpretation of relationships between mycoflora and allergic symptoms (e.g. Simoni et al. 2011; Jacobs et al. 2014). On the other hand, Dannemiller et al. (2014) demonstrated significant associations between low fungal diversity and childhood asthma development in a low-income, Mexican immigrant community in the USA. However, one characteristic of populations of Mexican descent is low asthma prevalence rates compared with other racial/ethnic groups in the USA (Lara et al. 2006).

DNA metabarcoding is a very promising approach to biodiversity investigations, and its effectiveness to recover the diversity present in mixed-species samples has been already tested for a range of organisms and environments, and even for poor-quality and low-quantity DNA (e.g. Taberlet et al. 2012; Bohmann et al. 2014; Valentini et al. 2015). However, there are also

potential limitations and difficulties that should be considered, such as errors during PCR and sequencing, quantitative assessment of different organisms, sequence coverage of reference databases (although fast improving), and species with incomplete lineage sorting for the barcode markers, which can lead to errors in identification. Yet, the increased and all the time improving precision obtained through DNA metabarcoding provides a highly potential tool for analysing, for example, indoor mycoflora. However, the full interpretation of even very accurate biodiversity results can be challenging. For instance, in the present study, we discovered that making explicit conclusions on the relationship between the indoor air quality and mycoflora is complicated by the occurrence of wide changes in spatial and temporal diversities and compositions among samples. In future DNA metabarcoding studies, a wider range of buildings, both with and without mould problems, should be investigated to allow deeper insights into the air quality issue of indoor space. In addition, the whole problem concerning fungi and other microbes in indoor air may be closely linked with decreases in the overall biodiversity and consequent alterations in the indigenous microbiota, and increased susceptibility to allergies. Evidence for the biodiversity hypothesis proposing a connection between biodiversity and allergic diseases has been provided in several recent investigations (e.g. Hanski et al. 2012; Ruokolainen et al. 2015).

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1

Figure captions

Fig. 1. Percentages (%) of sequences corresponding to filamentous ascomycetes, filamentous basidiomycetes, yeasts and other fungi among indoor fungi in five buildings, including two university office buildings, two nursery schools and a farmhouse, based on ITS2 sequences. Each pooled sample included sampling of both horizontal and vertical surfaces. Sequencing for January and July 2013 samples were conducted using 454 FLX pyrosequencing, while sequencing for January and July 2014 samples were performed using Illumina MiSeq sequencing.

Fig. 2. Principal component analysis (PCA) conducted for the pooled fungal samples, based on the ITS2 sequence data. University, nursery and farmhouse samples are marked with grey, black and white dots, respectively. U1 and U2 university samples from buildings 1-2, N1 and N2 nursery samples from buildings 1-2, F farmhouse sample; W13, S13, W14, S14, winter and summer samples from years 2013-14. Sequencing for January and July 2013 samples were conducted using 454 FLX pyrosequencing, while sequencing for January and July 2014 samples were performed using Illumina MiSeq sequencing.

Fig. 1.

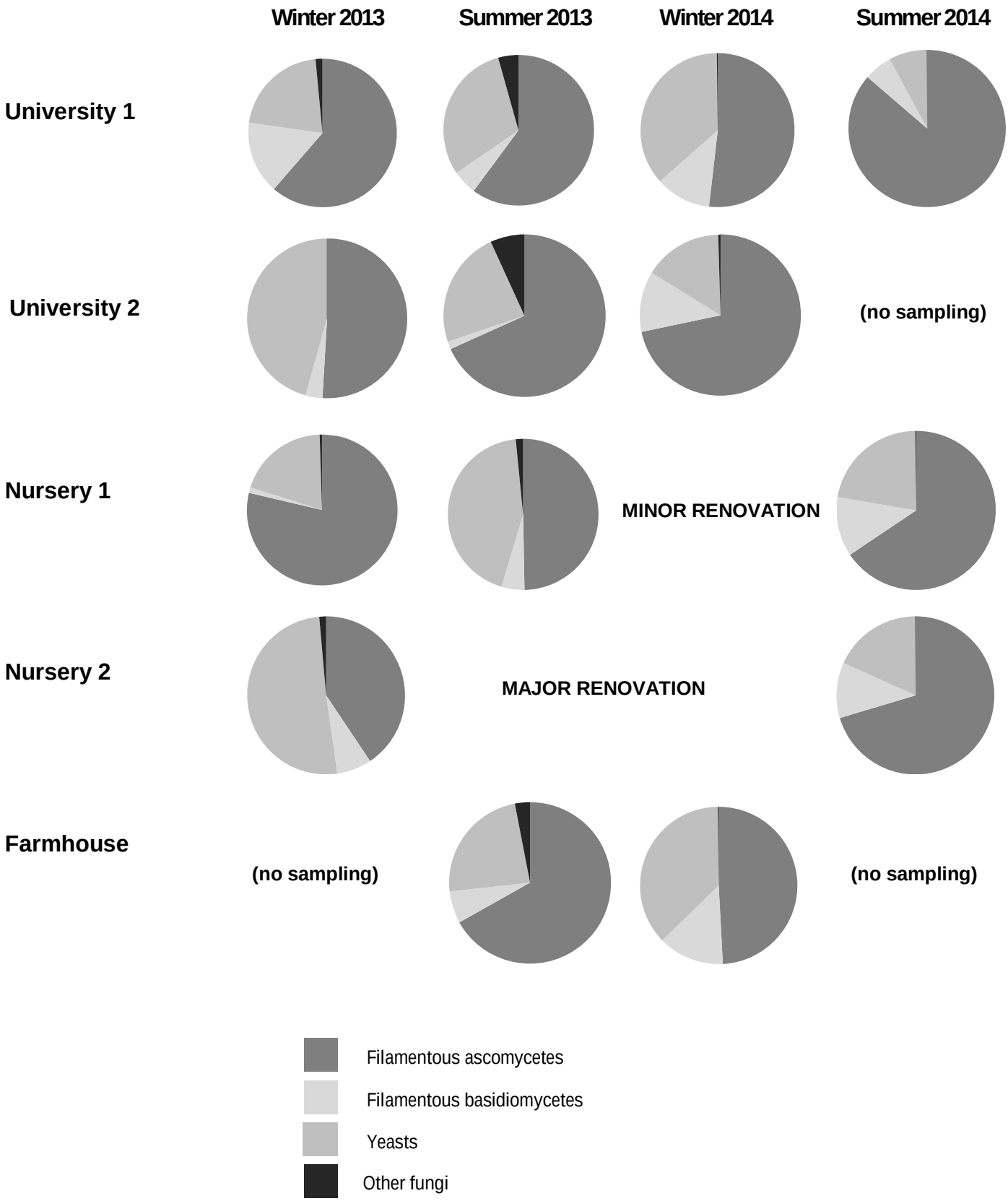
Winter 2013
Summer 2013
Winter 2014
Summer 2014
University 1
University 2
(no sampling)
Nursery 1
MINOR RENOVATION
Nursery 2
MAJOR RENOVATION
Farmhouse
(no sampling)
(no sampling)
Filamentous ascomycetes
Filamentous basidiomycetes
Yeasts
Other fungi

Fig. 2

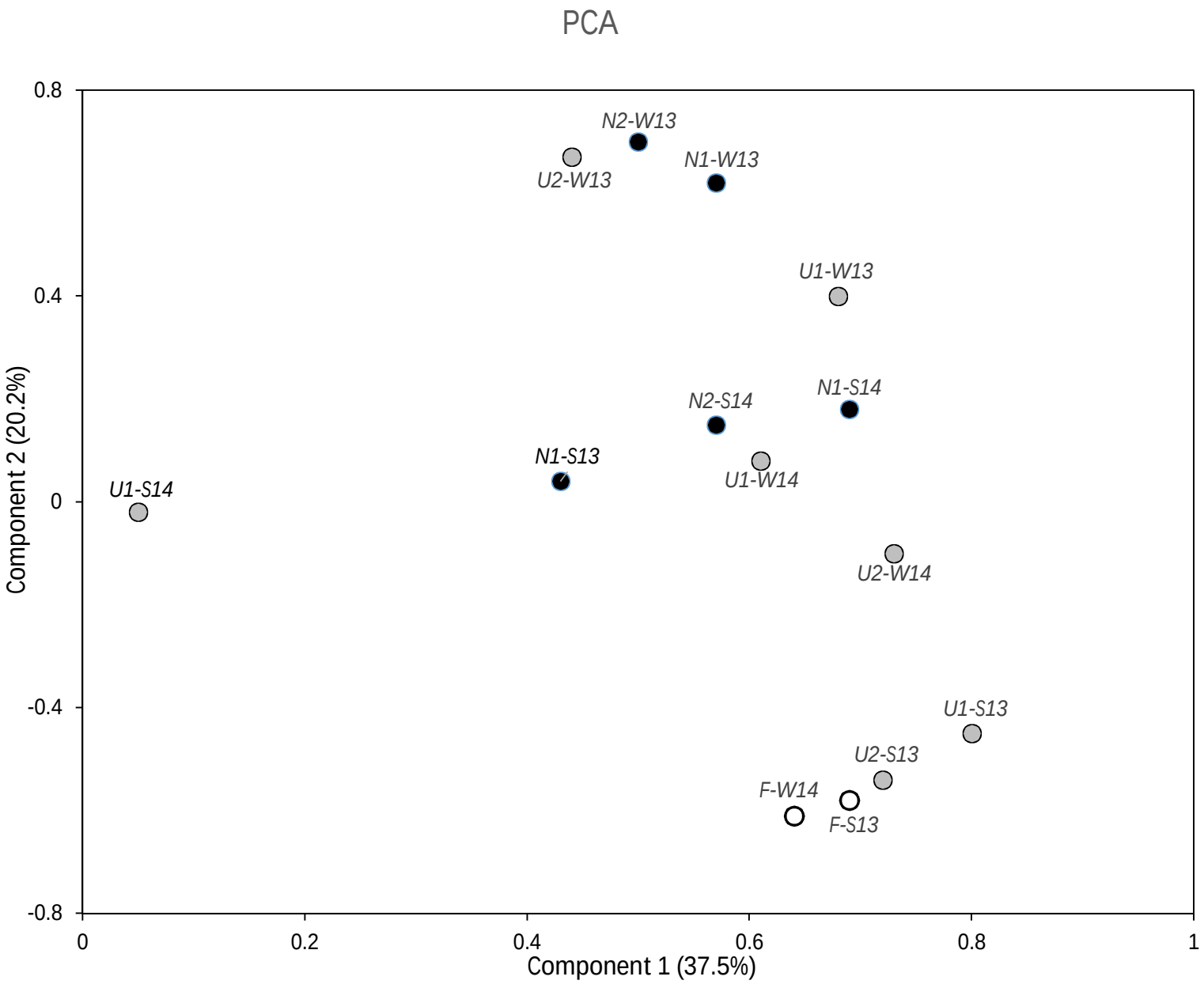
PCA
0.8
0.4
0
-0.4
-0.8
0
0.2
0.4
0.6
0.8
1
Component 2 (20.2%)
Component 1 (37.5%)
N2-W13
N1-W13
U2-W13
U1-W13
N1-S14
N2-S14
N1-S13
U1-W14
U1-S14
U2-W14
U1-S13
U2-S13
F-W14
F-S13

Table 1. Taxonomic diversity of indoor fungi at class, order, family and genus level in five buildings, including two university office buildings, two nursery schools and a farmhouse, based on ITS2 sequences. Each pooled sample included sampling of both horizontal and vertical surfaces. Range of variables among individual samples is given in parentheses. N, number of taxa; H, Shannon's diversity index.

		Winter 2013		Summer 2013		Winter 2014		Summer 2014	
Site		N	H	N	H	N	H	N	H
University 1	Class	19 (11-16)	1.95 (1.08-1.74)	21 (14-17)	2.08 (1.82-2.20)	16 (11-16)	1.62 (0.85-1.84)	15 (12-13)	0.47 (0.17-1.41)
	Order	55 (14-31)	2.66 (1.26-2.44)	57 (29-39)	2.80 (2.39-2.97)	47 (21-41)	2.12 (1.54-2.12)	48 (37-40)	0.65 (0.25-2.16)
	Family	90 (15-46)	2.98 (1.16-2.88)	97 (44-56)	3.30 (2.88-3.56)	82 (28-66)	2.47 (1.28-2.77)	88 (58-73)	0.82 (0.34-2.30)
	Genus	108 (26-61)	3.16 (1.43-3.10	143 (56-69)	3.59 (2.83-3.49)	146 (40-108)	2.73 (1.63-3.00)	165 (92-122)	0.87 (0.35-2.94)
University 2	Class	12 (8-10)	2.12 (1.70-2.12)	17 (10-17)	2.04 (1.84-2.04)	19 (14-18)	1.87 (1.66-1.87)	(no sampling)	
	Order	15 (10-12)	2.24 (1.87-2.10)	37 (16-33)	2.67 (2.43-2.57	46 (31-40)	2.57 (2.22-2.52)		
	Family	20 (10-13)	2.45 (1.80-2.16)	55 (16-47)	2.94 (2.49-2.84)	83 (46-71)	3.00 (2.38-2.92)		
	Genus	29 (17-18)	2.86 (2.25-2.54)	73 (27-60)	3.20 (2.96-2.98)	144 (72-111)	3.52 (2.62-3.46)		
Nursery 1	Class	19 (12-15)	1.95 (0.70-1.69)	18 (13-14)	1.92 (1.41-1.75)	(minor renovation, no sampling)		18 (15-17)	1.47 (1.23-1.42)
	Order	58 (22-33)	2.91 0.80-2.46)	54 (25-34)	2.86 (1.80-2.49)			58 (35-46)	2.47 (1.72-2.39)
	Family	97 (35-44)	3.22 (0.88-2.89)	109 (38-58)	3.25 (1.89-2.80)			118 (60-81)	2.80 (2.01-2.56)
	Genus	133 (50-81)	3.41 (0.78-3.42)	136 (46-80)	3.42 (1.74-3.17)			248 (108-151)	3.41 (2.22-3.05)
Nursery 2	Class	17 (6-17)	0.88 (0.13-2.06)	(major renovation, no sampling)		(major renovation, no sampling)		17 (11-13)	1.43 (1.40-1.41)
	Order	54 (14-45)	1.09 (0.17-2.81)					53 (28-36)	2.47 (1.97-2.42)
	Family	114 (20-83)	1.18 (0.18-3.40)					93 (35-62)	2.64 (2.00-2.58)
	Genus	176 (20-115)	1.18 (0.18-3.65)					170 (54-108)	3.07 (2.11-2.79)
Farmhouse	Class	(no sampling)		17 (12-17)	1.85 (1.36-1.95)	16 (14-16)	1.86 (1.68-1.99)	(no sampling)	
	Order			57 (25-40)	2.48 (1.64-2.67)	50 (28-45)	2.12 (1.93-2.15)		
	Family			104 (43-58)	2.92 (1.72-2.99)	102 (42-90)	2.44 (2.08-2.32)		
	Genus			158 (58-88)	3.01 (1.87-3.32)	198 (65-172)	2.95 (2.54-2.76)		

Table 2. Five most frequent fungal taxa and their proportions (%, in parentheses) in five buildings, including two university office buildings, two nursery schools and a farmhouse, based on ITS2 sequences. Each pooled sample included sampling of both horizontal and vertical surfaces.

Site	Winter 2013	Summer 2013	Winter 2014	Summer 2014
University 1	*Aureobasidium pullulans* (30.7) *Cryptococcus* (16.7) *Saccharomyces cerevisiae* (11.0) *Debaromyces hansenii* (8.7) *Cladosporium* (5.8)	*Cyberlindnera jadinii* (22.4) *Candida* (11.1) *Cladosporium* (10.8) *Penicillium* (6.2) *Aureobasidium pullulans* (5.0)	*Rhodotorula* (20.8) *Aureobasidium pullulans* (15.7) *Debaromyces hansenii* (12.9) *Candida* (10.0) *Caproventuria hanliniana* (6.1)	*Preussia* (85.7) *Penicillium* (3.0) *Aureobasidium pullulans* (1.8) *Cryptococcus* (1.7) *Pyrenophora* (0.9)
University 2	*Saccharomyces cerevisiae* (47.5) *Fusarium oxysporum* (14.1) *Malassezia* (7.7) *Capnobotryella* (3.6) *Rhodotorula* (3.3)	*Cyberlindnera jadinii* (23.7) *Candida* (11.7) *Exophiala* (6.7) *Cladosporium* (6.4) *Fontanospora* (5.3)	*Aureobasidium pullulans* (13.6) *Chaetomium* (7.5) *Cyberlindnera jadinii* (6.6) *Penicillium* (6.6) *Candida* (5.6)	(no sampling)
Nursery 1	*Cadophora* (46.3) *Saccharomyces cerevisiae* (11.9) *Aureobasidium pullulans* (6.0) *Cladosporium* (4.5) *Exophiala* (2.7)	*Pestalotiopsis* (20.1) *Lasiodiplodia* (14.4) *Saccharomyces cerevisiae* (8.8) *Aspergillus* (6.6) *Cyberlindnera jadinii* (6.5)	(minor renovation, no sampling)	*Aureobasidium pullulans* (20.3) *Cryptococcus* (13.5) *Candida* (9.2) *Rhodotorula* (4.7) *Saccharomyces cerevisiae* (4.2)
Nursery 2	*Saccharomyces cerevisiae* (45.4) *Cladosporium* (7.2) *Cryptococcus* (4.3) *Aureobasidium pullulans* (3.7) *Rhodotorula* (3.1)	(major renovation, no sampling)	(major renovation, no sampling)	*Cryptococcus* (16.6) *Aureobasidium pullulans* (13.8) *Chaetomium* (12.9) *Cladosporium* (6.7) *Leptosphaeria* (6.5)
Farmhouse	(no sampling)	*Cyberlindnera jadinii* (38.7) *Candida* (12.9) *Cladosporium* (6.5) *Malassezia* (5.7) *Aureobasidium pullulans* (5.1)	*Cyberlindnera jadinii* (20.9) *Candida* (15.9) *Aspergillus* (15.5) *Penicillium* (9.1) *Cryptococcus* (7.4)	(no sampling)